A Rapid and Accurate Quantitative Method for the Determination of Arachidic and Lignoceric Acids With Special Reference to Peanut Oil Analysis

By

CHAI-LAN YU, B.A., M.A.

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University

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I. INTRODUCTORY

1. GENERAL STATEMENT

Peanut oil is obtained by expressing or extracting the seeds of Arachis Hypogaea, L. Leguminosea. Although two decades ago its importance was not recognized, to-day it ranks third in the vegetable oil industry. In 1912, importation by the United States was only 7,626,000 pounds, but in 1919 it leaped to 87,000,000 pounds. China, a young champion in the peanut oil industry, has been cultivating the plant on a large scale for home consumption for more than two thousand years, and to-day is increasing her annual exports to this country and to the other parts of the world.

On account of its growing prominence in commerce, it has been long felt necessary to invent a more convenient method than the Renard-Tolman or its modifications, for a rapid and accurate determination of the characteristic acids in peanut oil in order to establish its genuineness and also its presence, as an adulterant, in other vegetable oils. The purpose of this research is to find such a method, based upon sound chemical principles, to study its optimum experimental conditions and to investigate sources and limits of errors, so that it will give consistent and reproducible quantitative as well as qualitative results.

2. HISTORICAL

Alexandre and Cadet,[1] in 1815, reported the striking hardness of soaps made from peanut oils. Thirty-nine years later, Gössmann,[2] in isolating the saturated acids by means of fractional precipitation with alcoholic and ammoniacal solutions of magnesium acetate and recrystallization of the solid acids from alcohol, discovered "arachidic acid," melting at 73°.5-75°C. In 1888, Kreiling,[3] through repeated recrystallizations, found, beside arachidic acid, another acid with a melting point of 81°. Its composition and properties proved it to be identical with the lignoceric acid discovered by Hell and Hermanns [4] in beechwood tar. Thus peanut oil contains two characteristic acid —arachidic and lignoceric acids, generally known under one name, "arachidic acid," or crude arachidic acid.

Since then many attempts have been made to isolate the crude arachidic acid as a reliable means of identifying peanut oil. In 1898, Jean [5] proposed a method based upon the comparatively low solubility of "potassium arachidate" at 15° in 90 per cent alcohol, previously saturated with the same soap. Bellier [6] in 1899 separated crude arachidic acid from peanut oil by crystallizing the insoluble acids from 70 per cent alcohol at 15°. This method was afterwards modified by Evers.[7] In 1914, Fachini and Dorta[8] suggested the potassium-soap-acetone method and two years later Kerr [9] proposed the magnesium-soap-alcohol method and two years later Kerr[9] proposed the magnesium-soap-alcohol method as qualitative tests for peanut

oil. But none of these are well known or have been shown to be of quantitative application.

The most popular and repeatedly investigated one is the "lead-ether" method. It owes its origin to Gusserow,[10] who was the first to observe that the lead soaps of unsaturated acids are much more soluble in ether than those of saturated acids of high molecular weights. In 1840, Varrentrapp [11] found Gusserow's method useful for the preparation of oleic acid. Since then various modifications have been made by Oudemans,[12] Kremel,[13] Röse,[14] Muter and de Koningh [15] and other investigators, but the essential principle, enunciated by Gusserow, remains the same.

As early as 1871, Gusserow's principle was introduced by Renard,[16] in peanut oil analysis. His technique was as follows: The fatty acids from the saponified oil were dissolved in 90 per cent alcohol to which was added a solution of lead acetate. The lead soaps were extracted with ether and the insoluble soaps decomposed under the same solvent with dilute hydrochloric acid. After evaporating off the solvent, "arachidic acid" was crystallized out from 90 per cent alcohol, through one night standing at 15° or 20°. The crystals were filtered off and washed first with 90 per cent alcohol and then with 70 per cent alcohol. The residue was dissolved with boiling absolute alcohol into a tared flask. The solvent was evaporated to dryness and the residue weighed. To this weight was added the amount of "arachidic acid" dissolved by 90 per cent alcohol in the filtrate and washings at the selected temperature.

By means of Renard's method, he and other investigators found that the yield of "arachidic acid," with melting points 71°-72° varied from 4.30 to 5.00 per cent and suggested the factor 20, i.e., the yield of "arachidic acid" multiplied by 20 should give the percentage of peanut oil. In 1891, de Negri and Fabris [17] studied known mixtures of olive oil and peanut oil by this method, finding it uncertain, when ten per cent or less of peanut oil was present.

Tortelli and Ruggeri [18] in 1898 showed that Renard's corrections for the solubilities of "arachidic acid" in 90 per cent alcohol were too low. They obtained from peanut oil 4.32-5.00 per cent of crude arachidic acid with melting points from 74°.3 to 75°.2. In the same year, Archbutt [19] also made a careful study of the application of the method to peanut oil analysis but could not reproduce the results of Tortelli and Ruggeri in all respects. Archbutt recommended a method of partial precipitation of lead soaps, which is a rather long procedure and of the same general principle as Renard's.

Renard's method was simplified by Tolman [20] in 1903. He specified 120 c.c. of 20 per cent lead acetate solution and omitted the decomposition of lead soaps under ether. He found the method collapsed in the presence of cottonseed oil or other oils possessing high contents of palmitic and stearic acids.

II. PRELIMINARY EXPERIMENTS

1. PREPARATION AND PURIFICATION OF FATTY ACIDS

Stearic Acid: Pure stearic acid can be obtained by saponifying beef tallow with alcoholic potassium hydroxide solution and then separating it from palmitic acid by fractional crystallizations from 95 per cent alcohol. It can be also obtained synthetically by hydrogenating unsaturated acids, containing eighteen carbon atoms, preferably pure oleic acid.

A specimen of technical stearic acid examined in the form of white blocks contained a small amount of acids of low molecular weights, especially palmitic acid. It melted at 50°-55° and had an acid number, 225.35.

A specimen of Kahlbaum reagent stearic acid melted at 68°-69° and had an acid number, 199.73. Since this was the easier of the two to purify, 50 grams were dissolved in 500 c.c. of boiling 95 per cent alcohol. The solution was set aside to crystallize. The crystals were filtered off and washed with the same solvent. A sample dried to constant weight at 50° showed upon analysis an acid number of 198.7 and no ash, nor iodine number. The crystals were again crystallized twice from the same strength of alcohol, washed and dried in the same manner. The resulting product melted at 69°.5, and had a molecular weight of 284.44. Complete analysis of three different samples thus purified and soaps prepared from them are as follows:

		Molecular weight	
Stearic Acid taken	KOH required	found	calcd.
1.3695 gm.	0.2697 gm.	284.44	284.39
2.3376	0.4487	284.46	
2.7630	0.5466	284.37	

Analysis of lead stearate:

Soap taken	Lead (found)	Lead (calcd.)
1.6055 gm.	26.73%	26.78%

Analysis of magnesium stearate:

Soap taken	Magnesium (found)	Magnesium (calcd.)
1.2669 gm.	4.12%	4.11%

Lignoceric Acid: Crude lignoceric acid was first obtained by the author from peanut oil by Renard-Tolman's method. On account of the unsatisfactory separation for the saturated lead soaps from the unsaturated and the expense and fire risk due to large quantities of ether, the author modified Jean's method and adopted the following shorter and more economical procedure, based upon the principle of the greater solubility of potassium oleate than those of stearate and lignocerate in 95 per cent alcohol,* at 25°, as shown below:

Soap used	Solid dissolved in 100 gm of 95% alcohol at 25°
Potassium stearate	0.64 gm.
Potassium lignocerate	0.15
Potassium oleate	41

*Density of alcohol = 0.80935 at 25°/4°.

Technique: About 300 c.c. of peanut oil were saponfied with one liter of saturated alcoholic potassium hydroxide solution by boiling half an hour under reflux condenser on a water bath. The solution was allowed to stand over night at about 10°. Then the insoluble soaps were then filtered off and washed with 95 per cent alcohol and decomposed with hot two-molar hydrochloric acid. After a clear, oily layer had formed cold water was poured into the flask. The solid acid shreds were filtered off and washed free from chloride. The dried acid cake was dissolved in hot 95 per cent alcohol and crystallized out twice from the same solvent.

These crystals melted at 75°-76° and showed molecular weights of 350-355; but no ash, nor iodine number. Upon repeated recrystallizations from absolute alcohol, a substance was finally obtained which melted at 79°-80° with a constant molecular weight of 368.1 (lignoceric acid—368.36). Its composition and properties were the same as those of Meyer, Brod and Soyka's isolignoceric acid.[21] The yield from 300 c.c. of peanut oil was about three grams.

The following soaps were prepared and analyzed:

Lead lignocerate taken	Lead (found)	Lead (calcd.)
1.3480 gm.	21.96%	21.99%
Magnesium lignocerate taken	Magnesium (found)	Magnesium (calcd.)
1.3161 gm.	3.22%	3.20%

Oleic acid: The acid can be prepared from tallow by the "lead-ether" method. Technical oleic acid would appear to be a promising raw material as source for the pure acid, but the specimen purchased by the author was so dark brown in color and full of sediment that it was deemed uneconomical to use it. Accordingly, a specimen of Kahlbaum reagent oleic acid was chosen as raw material. It was light straw colored and free from higher unsaturated acids.

This oleic acid was twice cooled to 10°, whereupon 8.6 per cent of solids were removed and proved to consist of palmitic acid and unsaponifiable matter. The filtrate had an acid number of 204.3 and iodine number 86.1 (Hanus).

Henceforth, the purification was carried out essentially according to Lewkowitsch's suggestion.[22] It was precipitated as lead oleate and extracted twice in small quantities with anhydrous ether. After filtering and distilling off the solvent, the residue was decomposed with five-molar hydrochloric acid in an electric vacuum oven. The acid liberated was washed nearly free from chloride in a separatory funnel and transferred to a beaker. To it was added an excess of half-molar barium hydroxide solution. Barium oleate was then filtered off and washed free from barium and chloride ions with distilled water. It was crystallized twice from 95 per cent alcohol. The crystals, after filtering and washing, were decomposed with five-molar hydrochloric acid in a vacuum oven. The oily layer was decanted into a separatory funnel and washed free from barium and chloride ions with distilled water. It was then extracted with anhydrous ether and transferred to a dry flask. The solvent was distilled

off at a low temperature and the residue put in a vacuum oven at 80° to remove traces of moisture, until constant weight was obtained.

The oleic acid thus purified gave the following analysis:

Ash	none
Molecular weight	282.65 (calcd.—282.37)
Iodine number (Hanus)	88.96 (calcd.— 90.07)

2. PREPARATION AND ANALYSIS OF SOAPS OF FATTY ACIDS

Lead and Magnesium soaps: Ten grams of the purified stearic or lignoceric acid were dissolved in 200 c.c. of 95 per cent alcohol by heating in a 800 c.c. beaker. To the clear solution a slight excess of half-molar aqueous solution of acetate of lead or magnesium was added. This mixture was boiled for twenty minutes with constant stirring. Three hundred cubic centimeters of distilled water were added and the whole allowed to stand at room temperature. The soap was then filtered off, washed free from the anion of the salt used and then extracted twice with 300 c.c. of redistilled 95 per cent alcohol under a reflux condenser and once with 200 c.c. of redistilled ether in the same way. When the mixture after each extraction had nearly come to room temperature the insoluble portion was filtered off and washed. Lead stearate was recrystallized from hot C. P. toluol in the form of large nacreous scales, while lead lignocerate, magnesium stearate and lignocerate were recrystallized from 95 per cent in crystals of smaller size. The residue, in each case, was dried in an oven at 80° to constant weight.

The oleates of lead and magnesium were prepared by double decomposition of their acetates with ammonium oleate, because the acetates of the respective metals do not react readily with oleic acid. Ten grams of oleic acid were rendered slightly alkaline to litmus paper by addition of molar ammonium hydroxide solution. After the addition of lead or magnesium acetate solution the mixture was heated to boiling with vigorous stirring for twenty minutes. Ammonium acetate and a small amount of ammonium oleate were leached away by repeated extraction with excess of warm water. The residue was filtered, washed and dried in a vacuum oven at 40°.

Analysis: Ashing. The sample was dried in a weighing bottle to constant weight. 1.0 to 1.5 gm. of the sample were weighed into a tared platinum crucible, except in the case of lead soaps, where quartz crucibles were employed. The covered crucible was supported by a quartz triangle and cautiously heated over a low, free flame. Melting and charring took place almost simultaneously. Towards the end of the incineration the flame was gradually increased, until a grayish white ash was obtained. Then it was heated to red heat, cooled in a desiccator and weighed. The residue was white and fluffy. The weight of ash from magnesium soaps represented an intermediary between the calculated weights of magnesium oxide and carbonate; from lead soaps between oxide and dioxide. Therefore, the author does not recommend the ashing method for analysis of such soaps.

The following procedure gave reliable quantitative results. The weighed sample was digested in a 300 c.c. covered porcelain casserole with an excess of concentrated sulphuric acid for about half an hour. It was then heated to fumes to remove the excess of sulphuric acid. The residue was taken up with a little water.

In the case of lead soaps the lead sulphate was filtered off on a Gooch crucible and determined as directed by Treadwell and Hall.[23] Magnesium sulphate solution was made slightly alkaline with ammonium hydroxide solution and precipitated, in presence of ammonium chloride, with ammonium orthophosphate and ignited to magnesium pyro-phosphate according to Schmitz's method:[24]

Analysis of Lead Soaps

	Soap taken	$PbSO_4$ (found)	Pb in lead soaps (found)	(calcd.)
Lead stearate	1.6056 gm.	0.6281 gm.	26.73%	26.78%
Lead lignocerate	1.3840	0.4449	21.96	21.99
Lead oleate	1.4293	0.5603	26.79	26.92

Analysis of Magnesium Soaps

	Soap taken	$Mg_2P_2O_7$ (found)	Mg in magnesium soaps (found)	(calcd.)
Magnesium stearate ..	1.2669 gm.	0.2313 gm.	4.12%	4.11%
Magnesium lignocerate	1.3161	0.1940	3.22	3.20
Magnesium oleate ...	1.6713	0.3147	4.10	4.14

Preparation of Potassium Soaps: Potassium soaps of the three acids were all prepared by titrating ten grams of the acid in 100 c.c. of 95 per cent alcohol with half normal alcoholic potassium hydroxide solution, using phenolphthalein as indicator. Since potassium stearate or lignocerate is sparingly soluble in cold 95 per cent alcohol, it was extracted with that strength of alcohol under a reflux condenser. After the mixture had come to 20°, the insoluble soaps were filtered off, washed and dried in an oven at 100°. Since potassium oleate is very soluble in the same strength of alcohol, there is no danger of occlusion of oleic acid. The solvent was evaporated off and the soaps dried in the usual manner.

Analysis: A sample of potassium soap was weighed out and digested with concentrated sulphuric acid as described before. After evaporation the residue was transferred to a tared platinum crucible, ignited at red heat, cooled and weighed as potassium sulphate. Slightly high results were obtained, as shown below:

	Soap taken	K_2SO_4 (found)	K in potassium soap (found)	(calcd.)
Potassium stearate ...	1.2044 gm.	0.3267 gm.	12.18%	12.13%
Potassium lignocerate.	1.6300	0.7006	9.64	9.61
Potassium oleate	1.0877	0.2980	12.29	12.19

3. DETERMINATION OF SOLUBILITIES OF FATTY ACIDS AND THEIR SOAPS IN VARIOUS SOLVENTS

The substances used for the determination of solubilities were all highly purified, as previously stated. The solvents were redistilled (alcohol over potassium hydroxide and silver oxide and ether over sodium, etc.), collecting the portion that came over at the proper boiling points, the first and the last fractions of the distillate being

rejected. Fifty c.c. of each solvent showed no residue upon evaporation to dryness.

Technique: An excess of the solid was placed in a 250 c.c. tightly stoppered bottle and covered with 150 c.c. of the solvent. A set of bottles so prepared was placed on a rotating platform machine and thus violently stirred over night at 25°. The bottles were then immersed in a water thermostat, maintained at 25° ± 0.005° for thirty-six hours. During the first twelve hours they were occasionally gently rotated, but left quiet after that, so that, at the end of thirty-six hours, a clear solution could be pipetted out. As a precautionary measure the tip of the calibrated pipette was covered with extracted filter paper. Twenty-five c.c. of the solution at 25° ± 0.005° were pipetted into a weighed 30 c.c. beaker. The solution was covered and slowly evaporated to dryness at a temperature much below the boiling point of the individual solvent. The residue in each beaker was dried in an oven at 80° to constant weight. The oleates were dried in a vacuum oven at 40° to avoid any possible oxidation.

In the meantime another portion of the solution was pipetted into a 25 c.c. specific gravity bottle. After twenty minutes' standing in the thermostat at 25° ± 0.005° it was wiped dry and weighed quickly.

After an additional one or two hours' standing another 25 c.c. portion of the saturated solution in the original bottle was pipetted, evaporated and the amount of dissolved solid determined as before. If this result did not check with the first one the whole experiment from the beginning was discarded and a new one performed, until a close check was obtained between two successive determinations, except in cases where the solubility was very high (like lead oleate in anhydrous ether or oleates of potassium and of magnesium in ethyl alcohol), where a close check was not found possible.

In any case, at least, a residue of two milligrams was considered to be final. If less than this amount was obtained additional 25 c.c. portions were successively evaporated and weighed, until a total of at least two milligrams was reached.

TABLE I

(a) Solubility of stearic acid in mixtures of water and ethyl alcohol:

Strength of Alcohol		Density of solution	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
Per cent by weight	Density 25°/4°	25°/4°		
91.53	0.80935	0.80755	1.80 gm.	2.28 gm.
86.16	0.82356	0.82163	1.01	1.24
63.07	0.87783	0.87725	0.087	0.099

(b) Solubility of lignoceric acid in mixtures of water and ethyl alcohol:

Strength of Alcohol		Density of solution	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
Per cent by weight	Density 25°/4°	25°/4°		
91.53	0.80935	0.80543	0.182 gm.	0.226 gm.
86.16	0.82356	0.82155	0.092	0.112
63.07	0.87783	0.87715	0.011	0.013

(c) Solubility of potassium stearate, lignocerate and oleate in mixture of water and ethyl alcohol:

Soap used	Strength of Alcohol Per cent by weight	Strength of Alcohol Density 25°/4°	Density of solution 25°/4°	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
Potassium stearate ...	91.53	0.80935	0.76746	0.483 gm.	0.640 gm.
Potassium lignocerate.	"	"	0.80625	0.123	0.153
Potassium oleate	"	"	0.86548	25.2	41.1

(d) Solubility of magnesium stearate in mixtures of water and ethyl alcohol:

Strength of Alcohol Per cent by weight	Strength of Alcohol Density 25°/4°	Density of solution 25°/4°	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
91.53	0.80935	0.79308	0.006	0.007
86.16	0.82356	0.82097	0.006	0.007
72.17	0.85821	0.85670	0.005	0.006
63.07	0.87783	0.87709	0.004	0.005
44.13	0.92275	0.92498	0.003	0.003
27.29	0.95792	0.95802	0.003	0.003
19.09	0.97122	0.97133	0.004	0.004
Water	0.99709	0.99602	0.004	0.005

(e) Solubility of magnesium lignocerate in mixtures of water and ethyl alcohol:

Strength of Alcohol Per cent by weight	Strength of Alcohol Density 25°/4°	Density of solution 25°/4°	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
91.53	0.80935	0.80701	0.005	0.006
86.16	0.82356	0.82133	0.005	0.005
72.17	0.85821	0.85780	0.006	0.006
63.07	0.87783	0.87695	0.004	0.005
44.13	0.92275	0.92420	0.003	0.003
27.29	0.95792	0.96072	0.002	0.002
19.09	0.97122	0.97102	0.002	0.002
Water	0.99709	0.99668	0.003	0.003

(f) Solubility of magnesium oleate in mixtures of water and ethyl alcohol:

Strength of Alcohol Per cent by weight	Strength of Alcohol Density 25°/4°	Density of solution 25°/4°	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
86.16	0.82356	0.83603	6.64 gm.	8.57 gm.
72.17	0.85821	0.85996	5.35	6.64
63.07	0.87783	0.88521	3.89	4.62

(g) Solubility of lead stearate, lignocerate and oleate in anhydrous ether:

Soap used	Density of solution 25°/4°	Dissolved solid in 100 c.c. of solution	Solid dissolved in 100 gm. of solvent
Lead stearate	0.70084	0.015 gm.	0.020 gm.
Lead lignocerate	0.70781	0.018	0.018
Lead oleate	0.73803	6.46	9.58

III. DISCUSSION OF "LEAD-ETHER" METHOD

Among all quantitative methods, as previously mentioned, the "lead-ether" method is certainly the best we have had and has been

very useful in the separation of saturated and unsaturated acids. Nevertheless, its use in peanut oil analysis is laborious and fraught with errors. From the solubility determinations we have learned that it is impossible to separate sharply and completely unsaturated acids from the saturated. Furthermore, in every 100 grams of anhydrous ether there is an inevitable loss of 0.020 gram of lead stearate and 0.018 gram of lignocerate, etc., as shown by the tabulations just given. The losses increase as the saturated acids decrease in molecular weights. Besides this the temperature, amounts of unsaturated soaps present and the quality and quantity of ether employed also have a marked influence upon the solubilities of lead soaps.

At temperatures above 20° a greater quantity of saturated lead soaps passes into solution. At too low a temperature the unsaturated soaps previously dissolved separate from the solution and deposit along with the saturated soaps. This behavior is particularly striking when separating the lead soap of erucic acid from those of saturated fatty acids.

The solubilities of lead soaps of saturated acids in ether are increased when a very large amount of unsaturated soaps is present, and conversely the presence of large amounts of unsaturated soaps results in a deposition of some of the unsaturated soaps with the saturated, even after thorough washings, as shown by the following instance:

Experiment	Stearic acid taken	Oleic acid taken	"Stearic" acid recovered	"Oleic" acid recovered
A	1.510 gm.	4.316 gm.	1.822 gm.	3.951 gm.
B	0.100	10.19	0.030	10.22

It is seen in experiment B that more than half of the lead stearate was dissolved in ether containing 100 times as much lead oleate, while, in experiment A, the undissolved lead stearate was contaminated with oleate as shown by an iodine number of 15.1.

It is also clear that different amounts of ether present as solvent will dissolve out different amounts of lead soaps. Since the solvent is very volatile, experience shows that it is extremely difficult to maintain, in different experiments, exactly the same amounts of ether, as specified by Renard-Tolman's method. Lewkowitsch[25] noticed that lead soaps are more soluble in anhydrous ether than in moist ether. In confirmation of his statement the author also found that if anhydrous ether is employed in the separation of known mixtures of lead oleate and lead stearate the amount of the stearic acid recovered is one or two per cent too low. On the other hand, in using ether containing a small amount of moisture the solid acid cake has an iodine number about 20 and its yield is several per cent higher than expected.

Experimental Difficulties: In the first place, the method as a whole is too long and sometimes tedious. Concluding from a great number of experiments, the following approximate length of time, not includ-

ing the setting up and taking down of apparatus, is required for successful operation in the first part of Renard-Tolman's method:

1. Saponification of oil, neutralization, transferring precipitation and boiling of lead soaps ..... one hour
2. Standing and cooling of boiled lead soaps ..... 1-2 hours
3. Filtering, washings of lead soaps with water and alcohol and their extraction with ether ..... 40 minutes
4. Standing and cooling of etheral solution of lead soaps ..... one night
5. Filtering and washing of insoluble lead soaps ..... 30 minutes
6. Decomposition of lead soaps with hydrochloric acid, filtering and washing of the solid acid cake ..... 1 hour

Total length of time ..... one night, five hours and ten minutes

In case of a large quantity of insoluble lead soaps a second decomposition is required, in order to get solid acids free from admixed soaps.

Secondly, filtration and washing of lead soaps is a difficult process. The washings come through very slowly, particularly when the quantity of soaps is large. In warm weather the solvent continues to evaporate and leaves behind, on the filter paper, as well as inside the funnel wall and stem, a coating of difficultly soluble soaps. A few cases have been met with where, owing to the formation of an emulsion, filtration was exceedingly slow.

Thirdly, the decomposition of lead soaps with dilute hydrochloric acid requires prolonged heating. Considerable care must be exercised to prevent bumping. When the amount of soaps is large, a second decomposition is necessary. The insoluble lead chloride formed and occluded by the solid acid cake is difficult to remove, necessitating repeated drainings with hot water.

Fourthly, the method is inadequate for the preparation of a large quantity of solid acids, if desired for research work or technical purpose. It not only consumes too much time and labor, involving fire risk and the expense of large amounts of ether, but also the separation is very incomplete. The solid acid cake obtained from saponifying more than 50 grams of peanut oil is soft and oily even after cooling for several hours.

For the reasons given the "lead-ether" method cannot give concordant or reproducible results in peanut oil analysis. With this method it has been stated by many investigators that peanut oil contains 3 to 5 per cent, or 4 to 5 per cent, of "arachidic acid" or crude arachidic acid. The new proposed method will, later on, prove that such wide variations are due to the errors in the "lead-ether" method itself. They are not statements of a fact.

IV. INVESTIGATION OF THE PROPOSED QUANTITATIVE METHOD

The author has systematically studied the quantitative behavior of 10 grams of pure soaps in 100 c.c. of various solvents and found, for instance, that no quantitative separation of sodium stearate and oleate or barium stearate and oleate could be effected in 90 per cent

alcohol at 20° or 25°. Zinc oleate was quite soluble in boiling ether, but the greater portion of it separated out when cooled to 15°. Thorium soaps behaved nearly the same as lead soaps. Lead stearate dissolved in hot toluol and upon cooling crystallized out in nacreous scales, but lead lignocerate, treated similarly, deposited in the form of a soft mass. Potassium soaps of saturated acids of high molecular weight were slightly soluble in 95 per cent alcohol at 15°, but they were too soft and gelatinous to be of any quantitative value.

Magnesium soaps in 90 or 95 per cent alcohol were found to afford a very satisfactory separation. At 25° 100 grams of 90 per cent alcohol dissolved only 0.007 gram of magnesium stearate, 0.006 gram of lignocerate but 8.57 grams of oleate. These solubilities diminish, as the temperature or concentration of alcohol decreases.

Peanut oil contains glycerides of oleic, linolic, palmitic, arachidic and lignoceric acids. It was assumed, in the following experiments, that the principle of the greater solubility of the magnesium soap of oleic acid in 90 per cent alcohol would apply to that of linolic acid and that the arachidate would have the same low solubility as stearate and lignocerate. This was afterward found to be true.

1. PREPARATION OF REAGENTS

(1) Alcoholic potassium hydroxide solution: Fifty grams of potassium hydroxide ("purified by alcohol") were dissolved in one liter of alcohol and filtered to remove impurities. This alcohol was the purified product obtained by distillation of 95 per cent alcohol that had been in contact with potassium hydroxide and silver oxide for one week. Fifty c.c. of this solution are more than sufficient for saponifying 10 grams of peanut, castor, corn, cottonseed, linseed, sesame, soya bean or tung oil.

(2) Alcoholic magnesium acetate solution: Fifty grams of magnesium acetate [$Mg(C_2H_3O_2)_2$] were dissolved in 100 c.c. of distilled water, heated to boiling and filtered. Three volumes of 95 per cent alcohol were then added to each volume of the cold filtrate.

(3) Alcoholic acetic acid: Twenty c.c. of glacial acetic acid were dissolved in 80 c.c. of 95 per cent alcohol. This reagent is quite stable, only slight esterification set in after a month's standing.

(4) Ninety per cent alcohol: Nine hundred c.c. of 95 per cent (by volume) alcohol were mixed with 50 c.c. of distilled water. Solutions so prepared from time to time varied in strength from 90.0 to 90.5 per cent by volume (densities 0.82473-0.82293 at 25°/4°).

(5) Seventy per cent alcohol: Seven hundred c.c. of 95 per cent alcohol were mixed with 250 c.c. of distilled water. The alcoholic solutions thus prepared also varied from 70.0 to 70.6 per cent by volume (densities 0.88117-0.87977 at 25°/4°).

2. STUDY OF EXPERIMENTAL CONDITIONS GOVERNING THE SEPARATION OF SATURATED AND UNSATURATED ACIDS IN KNOWN MIXTURES

(1) Influence of temperature upon the separation of magnesium soaps: Magnesium soaps have greater solubilities in warm 90 per cent alcohol than in cold. At 25° 100 grams of 90.3 alcohol (by volume) dissolves only 0.007 gram of magnesium stearate, 0.006 gram of magnesium lignocerate. At 35° more than 0.03 gram of stearate is dissolved, while at 10° it approaches a minimum. On the other hand, magnesium oleate, linolate and ricinoleate stay completely in solution at a temperature as low as 10°. Moreover, at temperatures above 20°, the small amount of unsaturated soaps absorbed by the saturated can be washed away more easily by means of 90 per cent alcohol than at lower temperatures.

Known mixtures of stearic or lignoceric acid with oleic acid were weighed out and neutralized with alcoholic potassium hydroxide solution. Magnesium soaps were precipitated by addition of excess of the alcoholic magnesium acetate reagent and allowed to stand over night at different temperatures. The insoluble soaps were filtered off and washed with 90 per cent alcohol, until the washings no longer gave a turbidity with water. They were decomposed with hot five-molar hydrochloric acid. The solid acid shreds were washed free from magnesium and chloride ions, dissolved in small amounts of absolute alcohol and quantitatively filtered into a 50 c.c. weighed beaker. The solvent was evaporated off, residue dried to constant weight at 80° and weighed.

Temperature	Stearic acid taken	Oleic acid taken	"Stearic" acid recovered	Iodine number of recovered "stearic acid"
25°	1.551 gm.	3.937 gm.	1.543 gm.	Almost zero
20°	1.549	3.948	1.567	3.5
10°	1.556	3.940	1.603	6.9

Temperature	Lignoceric acid taken	Oleic acid taken	"Lignoceric acid" recovered	Iodine number recovered "lignoceric acid"
20°	1.571 gm.	3.942 gm.	1.598 gm.	4.1

Examination of these figures reveals the fact that while a little of the stearic acid is lost at 25° the part recovered is pure as shown by zero iodine number, while at 20° and 10° practically all the stearic acid is recovered. At these temperatures, however, the recovered stearic acid is contaminated with a small amount of oleic acid, more so at 10° than at 20°, as indicated by the iodine numbers. For the present purposes it is better to recover all of the stearic, even though slightly contaminated, and consequently the lower temperatures are preferable for this separation.

(2) Influence of acidity and alkalinity: Only the solubilities of three normal magnesium soaps (magnesium stearate, lignocerate and oleate) have been determined with accuracy. Therefore, in separating magnesium oleate from magnesium stearate and lignocerate it

is essential to keep soaps as much in the form of normal salts as possible. Theoretically, normal soaps are formed with equivalent molar quantities of magnesium acetate and corresponding fatty acids. But in actual oil analysis, while neutralizing the soap solution, we are apt to make it a little too acid or alkaline.

In ascertaining the influence of a slight excess of acetic acid or potassium hydroxide upon the separation of magnesium stearate and oleate the first part of the experiment was carried out at 20° in the same manner as described above. The filtrate and washings from the solid acid cakes were collected, evaporated to small bulk and analyzed for magnesium according to B. Schmitz's method.[24]

Stearic acid taken	Oleic acid taken	Acid or alkali (alcoholic) added	"Stearic" acid recovered	Mg. from recovered "Stearic Acid" (found)	(calcd.)
1.513 gm.	3.917 gm.	5 c.c. of ½ M HAc	1.483 gm.	0.0629 gm.	0.0634 gm.
1.513	3.896	0.5 c.c.	1.507	0.0641	0.0646
1.513	3.889	Neutral	1.528	0.0665	0.0653
1.513	3.803	0.5 c.c. of ½ M KOH	1.601	0.0781	0.0684
1.513	3.909	3 c.c. of	2.105	0.1407	0.0900

From the foregoing data it appears that, on the acid side, there is either formation of free fatty acids or acid soaps. Since they are more soluble in the menstruum they are responsible for the loss of stearic acid. On the alkaline side the excess of magnesium comes from the precipitated magnesium hydroxide, the absorbed magnesium oleate and perhaps also the basic soaps. This experiment shows that it is safer to keep the soap solution slightly alkaline. This can be best attained by first neutralizing the alkaline soap solution with alcoholic acetic acid, using phenolphthalein as indicator and then titrating it back just to a permanent pink color.

3. STUDY OF EXPERIMENTAL CONDITIONS, GOVERNING THE SEPARATION OF THE SATURATED AND UNSATURATED ACIDS IN PEANUT OIL

Saturated magnesium soaps in peanut oil are almost entirely soluble in boiling alcoholic soap solution. But they separate out nearly completely upon standing at 10°, particularly magnesium stearate, arachidate and lignocerate. This complete separation, however, depends upon two important factors: (1) time of cooling and (2) amount of available magnesium acetate present in the solution. They again bear direct relationship to the amount of saturated glycerides in a given sample of peanut oil. From the quantities of solid acids obtained the amounts of these glycerides are unfortunately not very constant in peanut oils from different reliable sources:

Peanut oil sample	Saponification number	Iodine number	Solid acid obtained (corrected)
Virginia cold expression	187.5	90.7	15.6%
Spanish cold expression	187.0	94.1	20.0
Refined U. S. oil	190.3	91.6	13.5

The yields of solid acids from the first two oils are a little lower than those obtained by Dr. Jamieson and his co-workers[26] with the

"lead-ether" method, probably because of the greater solubility of magnesium palmitate in 90 per cent alcohol than that of lead palmitate in ether. But they do not mar the quantitative isolation of arachidic and lignoceric acids, as will be shown later.

(1) Time of cooling: The oil used in this and the next experiment was "Refined U. S. Peanut Oil," because it contained the smallest amount of saturated glycerides. The small amount of saturated magnesium soaps was easier to filter and wash. About 10 grams of the oil, accurately weighed, were saponified by a mixture of 50 c.c. of alcoholic potassium hydroxide solution and 50 c.c. of 95 per cent alcohol. The soap solution was neutralized with alcoholic acetic acid and titrated back just to permanent pink color. To this were added 50 c.c. of alcoholic magnesium acetate reagent. The mixture was allowed to stand in a refrigerator at 10° for different intervals. The insoluble magnesium soaps were filtered off, washed almost free from unsaturated soaps, as indicated by the production of only trace of turbidity in mixing the last part of the washings with water. Then the soaps were decomposed and analyzed for total solid acids, as shown below. It was found that one night standing is the best, producing coarser or more granular precipitate, which permits quick filtration and washing. It is also to be noticed that both, in this experiment and others of the same nature, the extent of thoroughness of washing has marked effect upon the magnitude of iodine number of the solid acids.

Time of cooling	Solid acids obtained	Melting point	Iodine number
2 hours	11.9%	60°-61°	4.3
4 hours	14.0	59°-60°	6.7
6 hours	14.2	58°-59°	7.1
Over night	14.1	59°-60°	6.3

(2) Amount of alcoholic magnesium acetate required for complete precipitation of saturated soaps in peanut oil: This series of experiments was carried out as above, except that, instead of using 50 c.c. of alcoholic magnesium acetate solution, various amounts of the reagent were added to the soap solutions. The mixtures were allowed to stand over night at 10°.

Oil taken	$MgAc_2$ solution (0.81 M.) added	Solid acids obtained	Melting point	Iodine number
10.24 gm.	None	3.9%	70°-71°	1.2
10.20	2 c.c.	6.7	65°-66°	3.5
10.52	5 c.c.	12.3	60°-61°	5.8
10.40	10 c.c.	14.2	58°-59°	6.3
10.23	20 c.c.	14.3	58°-59°	6.5
10.36	30 c.c.	14.2	59°-60°	6.3
10.23	40 c.c.	14.3	58°-59°	6.5

From the above table it is seen that 10 c.c. of the 0.81 molar alcoholic magnesium acetate solution are sufficient for this particular oil. In general, the strength of the reagent prepared at different times contains slightly different amounts of magnesium acetate and the solid acids from peanut oil also vary between 10 to 25 per cent. It is, therefore, safer to use 25 c.c. of the reagent for insuring a

complete precipitation of saturated soaps in peanut oils. In case, where the amounts of saturated magnesium soaps are high it is a great advantage to have just a partial precipitation of potassium oleate, linolate as magnesium oleate and linolate, because the former are more soluble and can be washed away more easily in the subsequent filtrations and washings.

V. DISCUSSION OF FATTY ACIDS CHARACTERISTIC TO PEANUT OILS

1. HISTORICAL

Genuine cold-drawn peanut oils contain, besides small amounts of free fatty acids and unsaponifiable matters like phytosterol, traces of moisture, etc., glycerides of water-insoluble acids. These insoluble acids constitute about 94 per cent of the original oil. Of this about 80 per cent are unsaturated acids—oleic and linolic acids. The rest are palmitic, stearic, arachidic and lignoceric acids. As to the exact amounts of individual acids, different investigators have differed considerably in their results.

Gössmann and Scheven,[27] Caldwells and Gössmann [28] and Schroeder [29] claimed to have found hypogaeic acid in peanut oils, but Schön,[30] Hazura and Grussner,[31] Bodenstein,[32] Meyer and Beer,[33] Heiduschka and Falser,[34] Jamieson, Baughman and Brauns [26] repeating their investigations, could not detect any hypogaeic acid. The presence of linolic acid in peanut oils was claimed by Hazura and Grussner [31] since they obtained its oxidation product, sativinic acid. This result was confirmed by Farnsteiner's isolation [35] and analysis of the tetrabromide of linolic acid. Farnsteiner calculated the unsaturated acids in peanut oil to consist of 30.3 per cent of oleic acid and 6 per cent of linolic acid, while Heiduschka and Felser maintained this to be 79.9 per cent of oleic acid and 7.4 per cent of linolic acid based on the weight of the oil. Farnsteiner's estimation of oleic acid is too low and Heiduschka and Felser's too high. Recently, Jamieson, Baughman and Brauns,[26] calculating from their dibromide and tetrabromide experiments, obtained 60.6 per cent of oleic acid, 21.6 per cent of linolic acid from the oil which they expressed from Spanish peanuts; and 52.9 per cent of oleic acid and 24.7 per cent of linolic acid in an oil obtained from Virginia peanuts.

Similarly, the amounts of palmitic, stearic, arachidic and lignoceric acids vary appreciably with different investigators as well as with the origin of peanut oils. Caldwells [36] first identified palmitic acid in peanut oil, but its presence was questioned by Kreiling.[8] Henner and Mitchell obtained 7 per cent of stearic acid, melting at 67°. Meyer and Beer [33] contended that it was a mixture of arachidic and lignoceric acids. However, the presence of palmitic and stearic acids in peanut oils have been quantitatively proved by more recent investigators; Heiduschka and Felser,[34] Jamieson, Baughman and Brauns,[26] but further researches to confirm their methods and data are still desirable.

Gössmann[2] discovered "arachidic acid" in peanut oil. Kreiling[3] proved it to be a mixture of arachidic and lignoceric acids. Heiduschka and Felser[24] isolated only 1.9 per cent of lignoceric acid and 2.3 per cent of arachidic acid. Jamieson, Baughman and Brauns,[25] calculating from methyl ester distillation, obtained 2.5 per cent of lignoceric acid and 3.3 per cent of arachidic acid from Virginia oil, and 3.0 per cent of lignoceric and 3.9 per cent of arachidic acid from Spanish oil.

2. SEPARATION OF ARACHIDIC AND LIGNOCERIC ACIDS IN PEANUT OILS

In 1871 Renard[16] used 90 per cent alcohol at 15° or 20° to separate arachidic and lignoceric acids from palmitic and stearic acids as a quantitative means of identifying peanut oil. To ascertain the validity of the method experiments were made by the author upon known mixtures of fatty acids. The acids were dissolved in 60 c.c. of hot 90.3 per cent alcohol (by volume) in a 150 c.c. covered beaker and allowed to stand over night in a thermostat at 20°. The insoluble acids were filtered off, washed twice with 10 c.c. of 90.3 per cent alcohol and then with 70.6 per cent alcohol (by volume), until the washings showed no turbidity with water. The residue was dissolved with absolute alcohol into a weighed platinum dish. The solvent was evaporated off and the residue dried to constant weight. The results are tabulated below:

Mixture of Stearic acid taken	Arachidic acid taken	"Arachidic acid" recovered	Molecular weight found	calcd.
0.3163 gm.	0.2078 gm.	0.1971 gm.	308.6	312.4

Stearic acid taken	Lignoceric acid taken	"Lignoceric acid" recovered	Molecular weight found	calcd.
0.3163	0.2394	0.2301 gm.	365.1	368.4

The losses shown by the figures above are due to the amounts of the acids dissolved by 90.3 per cent alcohol. They were found to increase or decrease, as the temperature is elevated or lowered. With application of solubility corrections a good quantitative separation can be made at 20° or 25° when the amounts of the interfering fatty acids present are not too large. Their solubilities are enhanced by the presence of small amounts of unsaturated acids. At 15° too much stearic acid is precipitated with the arachidic or lignoceric acid and the separation becomes impracticable.

3. CORRECTIONS FOR SOLUBILITIES OF ARACHIDIC AND LIGNOCERIC ACIDS IN 90.3 PER CENT ALCOHOL

Renard,[16] in 1871, first made solubility corrections by adding to the weight of "arachidic" acid, 0.0045 gram for each 10 c.c. of 90 per cent alcohol in the filtrate and washings at 20° or 0.0025 gram for each 10 c.c. at 15°. He apparently assumed that his weighed acid was a pure chemical compound and had, therefore, a definite solubility equilibrium at a given temperature and pressure, irrespec-

tive of the quantities of solid phase present in the saturated solution. In this he was mistaken. Tortelli and Ruggeri,[18] in 1898, showed that, firstly, Renard's corrections were too low and, secondly, at a given temperature the solubilities of the "weighed acids" in 90 per cent alcohol increased with the amounts of the mixed acids in the saturated solution. His data were confirmed by Archbutt.[19]

We have learned that the "weighed acid" is a mixture of arachidic and lignoceric acids, giving a melting point lower than that of either component. We have also learned that every 100 c.c. portion of saturated 90.3 per cent alcoholic solution at 25° dissolves 0.092 gram of lignoceric acid and 1.01 grams of stearic acid. Arachidic acid, as an intermediate member, has a greater solubility than lignoceric acid and lower solubility than stearic acid. Hence, with a 0.100-gram homogeneous mixture of arachidic and lignoceric acids no saturated solution can be formed at 25°. With a 0.200-gram mixture saturation takes place. If it is a binary system any increase in the solid phase from now on under the same condition would not alter the solubility equilibrium, but the system is ternary, having three components—arachidic acid, lignoceric acid and alcohol. There are two solid, one vapour and one liquid phases. Hence the system is univariant, according to the formula:

$$P + F = C + 2,$$ where P is the number of phases
F degrees of freedom and
C the number of components.

As the temperature and pressure are fixed in the author's experiments, change of the quantities of the two solid phases will alter the equilibrium. As the increase in the homogeneous mixture is constant, the increase in the solubility will also have a constant value. This is warranted by experimental results:

TABLE IIa

Solubilities of arachidic and lignoceric acid mixtures (melting point 72°-73°) in 90.3 per cent* alcohol at 25°:

Mixture taken	Dissolved solid in 100 c.c. of solution
0.600 gm.	0.172 gm.
0.500	0.162
0.400	0.152
0.300	0.144
0.200	0.132
0.100	No saturated solution is formed

*Strength of alcohol described in IV, 1.

TABLE IIb

Solubility of arachidic and lignoceric acid mixtures (melting point 72°-73°) in 90.3 per cent alcohol at 20°:

Mixture taken	Dissolved solid in 100 c.c. of solution
0.600 gm.	0.099 gm.
0.500	0.091
0.400	0.083
0.300	0.075
0.200	0.068
0.100	0.058

TABLE III

(a) Solubility corrections for the weighed acid mixtures (melting point 71°-73°) per 100 c.c. of the alcoholic solution at 20°:

Weight of acid mixture obtained	Solubility correction
0.60 gm.	0.099 gm.
0.55	0.096
0.50	0.091
0.45	0.087
0.40	0.083
0.35	0.079
0.30	0.075
0.25	0.070
0.20	0.066
0.15	0.062
0.10	0.059
0.05	0.054

(b) Solubility corrections for the same at 25°:

Weight of acid mixture obtained	Solubility correction
0.60 gm.	0.172 gm.
0.55	0.167
0.50	0.162
0.45	0.157
0.40	0.152
0.35	0.147
0.30	0.142
0.25	0.137
0.20	0.132
0.15	0.127
0.10	0.122
0.05	0.117

The solubility corrections in the above table were computed from Tables IIa and IIb. The corrections would appear to be too small for actual peanut oil analysis, since the "weight of acid mixtures obtained" really represents the "acid mixture taken" in Tables IIa and IIb. But we must consider the following facts: (1) Solubility corrections in Tables IIa and IIb were carried out with arachidic and lignoceric acid mixture alone. In peanut oil analysis the 60 c.c. of 90 per cent alcoholic solution contain considerable quantities of palmitic and stearic acids, which undoubtedly inhibit the solubilities of arachidic and lignoceric acids. (2) The solubility corrections in Table III were determined with exactly 90.3 per cent (by volume) alcohol. In practical work, due to loss of alcohol vapour through incipient boiling and slight evaporation upon standing, the final strength of alcohol in the beaker is, strictly speaking, less than 90 per cent. Since solubilities of saturated fatty acids are functions of concentration of alcohol the solubilities of arachidic and lignoceric acids must have been diminished. So, for practical purposes, the given corrections represent, perhaps, a state nearer to truth than we would otherwise obtain.

VI. TECHNIQUE OF THE PROPOSED QUANTITATIVE METHOD

About 10 grams of oil, actually weighed, are saponified in a 300 c.c. Erlenmeyer flask by a mixture of 50 c.c. of alcoholic potassium hydroxide solution and 50 c.c. of 95 per cent alcohol. The saponification is carried out by heating for half an hour under a reflux condenser.

Upon the completion of saponification the soap solution, while still warm, is neutralized with the alcoholic acetic acid reagent, using phenolphthalein as indicator. Thereupon just enough alcoholic potassium hydroxide solution is added to give a permanent pink color. Twenty-five c.c. of alcoholic magnesium acetate reagent are added. This mixture is heated just to boiling and allowed to stand over night in a refrigerator at about 10°. The insoluble soaps are then filtered off and washed with 30 c.c. of 90 per cent alcohol by first rinsing out the soaps adhering to the flask.

The greater part of the filtered and washed magnesium soaps are then transferred to the 300 c.c. Erlenmeyer flask. One hundred c.c. of boiling five-molar hydrochloric acid are poured over the pierced filter paper and the filtrate is caught in the same flask. The magnesium soaps are decomposed by boiling for about five minutes. After a short standing cold water is poured into the flask slowly, so as to produce shreds of solid acids, which are filtered off and washed free from magnesium and chloride ions, as indicated respectively by alkaline ammonium orthophosphate and silver nitrate tests. The moisture in the flask is removed by drying at 80°-100°. The solid acids are transferred to a 150 c.c. beaker. Sixty c.c. of 90 per cent alcohol* are warmed in three successive portions in the flask and poured over the filter paper to dissolve out completely the remaining acid shreds and to catch the filtrate in the beaker containing the acids previously transferred. The covered beaker is allowed to stand over night in a thermostat at 20° or 25°. The crystals which have separated are filtered off and washed twice with 10 c.c. of 90 per cent alcohol and then with 70 per cent alcohol* (the temperature of 70 per cent alcohol washing should be about 20°), until ten drops of the washing produce no turbidity with water. The filtrate and washings from 90 per cent alcohol are collected and accurately measured. The residue is transferred into the original beaker and dissolved with small portions of absolute alcohol. The solution is carefully poured over the filter paper and the filtrate is caught in a weighed platinum dish or a weighed 50 c.c. beaker. The original beaker and filter paper are quantitatively washed with another small portion of absolute alcohol. The solvent is evaporated off over an electric hot plate at about 70° or over a water bath. The residue is dried to constant weight at 80°. This weight is corrected for the total amount of the mixture of arachidic and lignoceric acids dissolved in the filtrate and washings of 90 per cent alcohol at the

* For exact strengths and densities, see Section IV, Subdivision 2.

selected temperature. The mixed acids thus obtained melt between 71° and 73°. Upon analysis they show no ash, no iodine number and a mean molecular weight of about 340. Upon recrystallization from another 60 c.c. portion of 90 per cent alcohol the mixed acids melt at 75°-76° or occasionally slightly higher. This recrystallization is valuable as a confirmatory test.

VII. APPLICATION OF THE PROPOSED METHOD TO PEANUT OIL ANALYSIS

1. ANALYSIS OF PURE PEANUT OIL

(1) Unrefined but authentic peanut oils.

TABLE IV

Oil sample	Oil taken	Acids found	Correction for acids dissolved at 25°	—Total acids— gm.	Per cent
Spanish	10.40 gms.	0.4743 gm.	+ 0.0960(60cc)gm.	= 0.5703	5.48
Texas (Delion).	10.45	0.4695	+ 0.1035(65cc)	= 0.5728	5.47
Java	10.21	0.4335	+ 0.1089(66cc)	= 0.5424	5.41
Virginia	10.80	0.4843	+ 0.0912(57cc)	= 0.5755	5.31
Alabama	10.14	0.4232	+ 0.1088(66cc)	= 0.5318	5.24
Mexican	10.04	0.4281	+ 0.0977(64cc)	= 0.5257	5.23
China (Canton).	10.35	0.4404	+ 0.0952(61cc)	= 0.5356	5.17

The above crude oils were directly expressed from peanuts without undergoing any refining. They must not be confounded with commercial crude oil, meaning impure or adulterated oils. The above oils represent nearer the true contents of arachidic and lignoceric acids than refined oils. With the exception of China crude, expressed by the Chinese fashion in China, the remaining six oils do not differ more than 0.25 per cent in their arachidic and lignoceric acid contents. By taking into consideration the experimental errors and variations in expressing and handling of these oils by different persons, the difference in the contents of the two acids among these oils as given in Table IV is surprisingly small.

It is of interest to note that the Texas crude oil came from hot expression, while the rest are all of cold expressions. The Texas crude was dark brown in color and contained an abundance of suspended peanut solids. The impurities were removed by centrifuging previous to analysis.

Analysis of Texas crude oil yielded 4.92 per cent and 5.47 per cent of arachidic-lignoceric acid mixture before and after centrifuging respectively.

(2) Analysis of three unrefined genuine oils were also carried out at 20° to show the influence of change of temperature upon the final result during the crystallization of arachidic and lignoceric acids from 90 per cent alcohol.

TABLE V

Oil sample	Oil taken	Acids found	Correction for acids dissolved at 20°	—Total acids— gm.	Per cent
Spanish	10.26 gms.	0.5010 gm.	+ 0.0611(66cc)gm.	= 0.5621	5.47
Virginia	10.40	0.4949	+ 0.0573(63cc)	= 0.5522	5.30
Chinese (Hongkong) .	10.90	0.4771	+ 0.0570(64cc)	= 0.5341	4.89

(3) Analysis of commercial refined peanut oils. These samples were procured from reliable brokers, oil expressing and refining companies. The analyses were performed in the same way as the aforesaid seven crude oils. From the following table it is noticed that processes of refining entail a small loss of arachidic and lignoceric acids:

TABLE VI

Oil sample (10 gm. in each case)	Total of arachidic and lignoceric acids found
Deodorized oriental peanut oil—China	5.13%
Refined peanut oil	5.01%
Refined U. S. peanut oil	4.98%
Refined Virginia peanut oil	4.78%
Refined Canton peanut oil	4.79%

Judging from above analyses (Tables IV, V, VI), an average sample of genuine peanut oil contains about 5.0 per cent of arachidic and lignoceric acid mixture. With any amounts below 4.5 per cent the sample can be justly pronounced adulterated or impure. Hence, for practical purposes, the factor 20, first suggested by Renard,[16] can be conveniently retained.

(4) Analytical results obtained with the "lead-ether" method (Renard-Tolman): The inherent experimental errors and difficulties of this method have been fully discussed in Section III. It is not our expectation that this method should be capable of giving consistent results. With Renard's method and Tortelli and Ruggeri's modifications,[18] Archbutt,[19] using both ordinary and recrystallized lead acetate, obtained percentages of the mixed acids varying from 4.3 to 5.1 per cent with melting points between 70° and 73° with the same sample of peanut oil. With different samples of peanut oils Tolman and Munson[33] obtained 3.41 to 4.24 per cent, having melting points between 72° and 73°. Heiduschka and Felser[34] obtained 4.74 to 5.66 per cent with melting points varying from 67° to 69°. The author also found that two experiments with the same sample of oil and under conditions as identical as possible gave results differing about 0.3 per cent at 20°, whereas those from the proposed method do not differ more than 0.05 per cent at the same temperature. Experiments performed by the "lead-ether" method on different days give greater variations. The following are the comparative analyses by the "lead-ether" method:

Oil	Oil taken	Acids found	Correction for acids dissolved at 20°	—Total acids— gm.	Per cent
Spanish	10.40 gms.	0.5015 gm.	+ 0.0573(65cc)gm.	= 0.5588	5.37
Virginia	10.93	0.4661	+ 0.0660(75cc)	= 0.5321	4.89
Chinese	10.50	0.4542	+ 0.0450(55cc)	= 0.4992	4.75

(5) Analysis of commercial crude and adulterated oils:

	Sample	Acids found	Correction for acids dissolved at 25°	—Total acids— gm.	Per cent
(1)	10.35 gms.	0.3542 gm.	+ 0.0867(59cc)gm.	= 0.4319	4.17
(2)	10.74	0.2380	+ 0.0952(70cc)	= 0.3332	3.10

Sample (1) was afterwards admitted by the dealer to be a mixture

of crude peanut oil and other oils. Sample (2) is, however, sold as refined peanut oil for table purposes. In using the factor 20 it follows that it contains only 60 per cent of peanut oil. Since it gave a positive Halphen reaction it is evident that it contains cottonseed oil.

2. STUDY OF MIXTURES OF PEANUT OILS WITH OTHER OILS

Generally, in oil analysis, the chemist is obliged to rely upon the determination of the so-called "constants" or numbers, such as saponification number, iodine number, etc., to establish the purity or adulteration of a fatty oil. These constants are very valuable when applied to one oil or a mixture of not more than two oils with widely different constants, but a skillful adulterator may, by use of two or more than two adulterants, perpetrate a fraud which these numbers will fail to reveal. It is evident, however, that such fraud can now be definitely detected quantitatively, as well as qualitatively, by the proposed method whenever peanut oil is either adulterated or used as an adulterant of other more expensive oils, as shown in accompanying Tables VII, VIII, IX.

Common adulterants of peanut oils are cottonseed, rape, sesame and poppyseed oils, and Lucas[89] reported that nitrobenzene has been found in peanut oil sold in Porto Rico. This adulteration was for the purpose of making peanut oil appear to be oil of bitter almonds. During the European war, when the supply of olive oil in this country became scarce, cold-drawn peanut oil was used as adulterant and also as a substitute for olive oil. When the demand for peanut oil became great cold-drawn cottonseed oil crept in as an adulterant of peanut oil. This condition would appear again at any moment when the demand for olive oil is greater than its supply.

French olive oil and American cold-drawn cottonseed oil were analyzed, both singly and admixed with pure peanut oil, to determine the validity of the proposed method. These oils had the following properties:

Sample	Specific gravity	Saponification number	Iodine number	Solid acid content (corrected)
French olive oil.....	0.916(20°/4°)	195.2	84.0	11.6%
American cottonseed oil	0.921(20°/4°)	196.5	106.3	19.6

Since genuine peanut oil contains about 5.0 per cent of the characteristic acids, this method, as well as the "lead-ether" method, would not be expected at 20° to detect less than 10 per cent of peanut oil in a mixture if one started with only 10 grams of oil for analysis. In order to increase the sensitiveness of the method, a larger sample of oil mixture must be taken. Hence, in studying the known mixtures of oils, experiments were first carried out with the different amounts of the olive and cottonseed oils to see whether their solid acids would interfere:

Olive oil taken	90% of alcohol used to dissolve solid acids	Crystallization of solid acids at 20°
10 gm.	60 cc	None
30	60 cc	"
50	100 cc	"

Cottonseed oil taken	90% of alcohol	Crystallization of solid acids at 20°
10 gm.	60 cc	None
20	100 cc	"
30	100 cc	"
50	150 cc	"

(1) Analysis of prepared mixtures of French olive oil and Virginia crude peanut oil.*

TABLE VII

Sample taken	Composition of mixture Peanut	Olive	Mixed acids found	Correction for mixed acids dissolved at 20°	Total acids	Peanut oil found
10 gm.	100%	—	0.4769 gm.	+ 0.0535(57cc) gm.	= 0.5304 gm.	100%
10	90.0	10.0%	0.4280	+ 0.0576(67cc)	= 0.4856	91.7
10	50.2	49.8	0.2240	+ 0.0456(68cc)	= 0.2606	49.2
10	20.1	79.9	0.0620	+ 0.0388(68cc)	= 0.1008	19.0
30	10.0	90.0	0.1263	+ 0.0354(60cc)	= 0.1617	10.1
50	5.9	94.1	0.0508	+ 0.0568(97cc)	= 0.1076	4.1

*The factor for this particular peanut oil was known to be 18.9.

(2) Analysis of prepared mixtures of American cottonseed oil and Spanish crude peanut oil.**

TABLE VIII

Sample	Composition of mixture Peanut	Olive	Mixed acids found	Correction for mixed acids dissolved at 20°	Total acids	Peanut oil found
10.0 gm.	100%	—	0.4891 gm.	+ 0.0615(64cc) gm.	= 0.5506 gm.	100%
10.0	90.3	9.7	0.4363	+ 0.0559(65cc)	= 0.4922	89.4
10.0	50.1	49.9	0.2260	+ 0.0483(70cc)	= 0.2743	49.9
10.0	20.0	80.0	0.0642	+ 0.0414(74cc)	= 0.1056	19.2
30.0	10.9	79.1	0.1101	+ 0.0597(97cc)	= 0.1698	10.3
50.0	5.6	94.4	0.0350	+ 0.0742(141cc)	= 0.1092	3.9

**The factor for this particular peanut oil was known to be 18.2.

(3) Analysis of prepared mixtures of American cottonseed oil with refined U. S. peanut oil.***

TABLE IX

Sample taken	Composition of mixture Peanut	Cottonseed	Mixed acids found	Correction for mixed acids dissolved at 25°	Total acids	Peanut oil found
10.0 gm.	100%	—	0.4086 gm.	+ 0.0896(60cc) gm.	= 0.4982 gm.	100%
10.0	90.2	9.8	0.3452	+ 0.1029(70cc)	= 0.4481	90.1
10.3	50.0	50.0	0.1709	+ 0.0740(60cc)	= 0.2449	49.2
10.0	40.9	59.5	0.1140	+ 0.0860(70cc)	= 0.2000	40.2
10.6	30.7	69.3	0.0720	+ 0.0738(62cc)	= 0.1458	29.2
20.0	20.1	79.9	0.0566	+ 0.110 (93cc)	= 0.1666	16.2

***The factor for this particular peanut oil was known to be 20.1.

The above three peanut oils contained three different contents of arachidic and lignoceric acids, that is 5.30 per cent for Virginia crude oil, 5.49 per cent for Spanish crude oil and 4.98 per cent for the U. S. refined oil. Hence, in order to test the accuracy and validity of the proposed method, the three respective factors, 18.9, 18.2 and 20.1 in Tables VII, VIII and IX, were used instead of the

general factor 20. Using the general factor 20, we will find that the maximum deviation of the percentages found from those in the prepared mixtures is not more than 5 per cent, except three cases in the first two tables. It is therefore understood for practical purposes, using the average factor of 20, that an allowance for a possible error of 5 per cent should be made in the determination of crude or refined commercial peanut oils.

5. DISCUSSION OF LIMITS OF ERRORS IN THE EXPERIMENTAL RESULTS

The foregoing tabulated data of arachidic and lignoceric acids from pure peanut oils and their prepared mixtures are accurate and reproducible. The proposed method gives a higher percentage and more consistent yields than the "lead-ether" method or any other current method. Solid acids from common vegetables—oils like refined castor, corn, cottonseed, linseed, olive, rape, sesame, soya bean and tung oils—do not give any precipitation in 90 per cent alcohol upon standing over night in a thermostat at 25°. At 20° rape oil is an exception, giving about 0.5 per cent of the insoluble acids, melting at 70°.

Experimental errors involved in the slight alterations of working conditions are very small and negligible. Experiments were carefully carried out with the same sample of peanut oil but with the following alterations: (1) At different days. (2) About 10 c.c. more or less of 95 per cent alcohol than the amount specified in saponifying the oil was used. (3) Prolonged cooling of the magnesium soap solution. (4) Slight excess of alcoholic acetic acid or slight alcoholic potassium hydroxide solution during neutralization. (5) Crystallization of the arachidic and lignoceric acids at 20° or 25°. (6) Washing of the mixed acid with 70 per cent alcohol at one degree higher or lower than 20°. These slight alterations do not affect the final results to any appreciable extent. Some values obtained on a given sample at different times and temperatures are shown below:

Spanish Crude Peanut Oil

Date of experiment	Temperature of crystallization of mixed acids	Total mixed acids found
Oct. 26, 1921	25°	5.49%
Oct. 25	25°	5.45
Nov. 1	20°	5.48
Nov. 1	20°	5.50
Nov. 10	25°	5.41

Refined U. S. Peanut Oil

Date of experiment	Temperature of crystallization of mixed acids	Total mixed acids found
Oct. 7, 1921	25°	5.00%
Oct. 7	25°	4.97
Nov. 1	20°	5.01
Nov. 6	25°	4.87
Nov. 6	25°	4.99

From the above data, the mean errors of the mean were computed from the equation of least squares—$m = \sqrt{\frac{\Sigma d^2}{n(n-1),}}$ where

m = mean error of the mean,
Σd^2 = square of the total deviation from the mean,
n = number of determinations.

The mean errors of the mean for Spanish crude peanut oil and refined U. S. peanut oil were calculated to be ± 0.03 and ± 0.04. The percentage errors of the average deviations from the mean are ± 0.51 per cent for Spanish crude and ± 0.76 per cent for refined U. S. oil.

Constant errors in the proposed method are (1) Loss of arachidic and lignoceric acids due to the slight solubilities of their magnesium soaps in 90 per cent alcohol and (2) Loss due to the solubilities of these two acids in 90 per cent alcohol at 20° or 25° during their crystallizations. In the first case, the loss is theoretically less than 0.02 gram. The loss in the second case is corrected by the solubilities as determined and shown in Tables IIa and IIb.

VIII. EXTENSION OF THE PROPOSED METHOD

1. SEMI-QUANTITATIVE TEST FOR PEANUT OIL FROM THE TITRATED SOLUTION FROM SAPONIFICATION NUMBER

Since the saponification and iodine numbers are always determined in oil testing, great advantage would be gained by saving the trouble of extra weighing and saponifying for a specific test of peanut oil, particularly when merely a qualitative determination is desired, if a use could be devised for the titrated soap solution from the saponification number. Since lead chloride or sulphate are insoluble in organic solvents and extraction with ether is bothersome, nothing along this line of investigation has been attempted with the "lead-ether" method, but tests have shown such to be possible with the new proposed method.

After the saponification "number" titration, 25 c.c. of alcoholic magnesium acetate reagent are added. The mixture is allowed to stand over night at 10°. The insoluble magnesiumm soaps are filtered off and henceforth the experiment is carried out at 20° or 25° in the same manner as previously described for the quantitative procedure. Some experimental results are given below:

Sample	Oil taken	Saponification number	Total mixed acid found	Total acids (semi-quantitative)	Total acids (quantitative)*
Spanish crude..	5.765 gm.	187.0	0.2703 gm.	4.86%	5.49%
Virginia	4.671	186.4	0.2230	4.77	5.30
Refined U. S....	5.106	191.1	0.2355	4.61	4.98

In comparing the last two columns, the semi-quantitative method

* Compared with Tables IV and VI.

is only about 10 per cent less accurate than the data from quantitative procedure of the proposed method. In commercial analysis, where speed is more essential than accuracy, this affords a very convenient means for the approximate determination of peanut oil. In this shortened procedure the factor 22 should be used.

2. QUALITATIVE TESTS FOR RAPE OIL AND TUNG OIL

(1) Rape Oil: In testing the proposed method with the more common vegetable oils, it was found that olive and sesame oils both gave about 10 per cent of solid acids, corn oil about 8 per cent, soya bean oil less than 5 per cent, linseed and castor oils mere traces. The solid acids were obtained by decomposing the washed insoluble magnesium soaps, but since they are completely soluble in 90 per cent alcohol they do not affect the quantitative determination of peanut oil. Besides cottonseed oil, rape oil also gave a large quantity of insoluble magnesium soaps upon standing over night at 10°. After decomposition of the magnesium soaps from rape oil and dissolution of the solid acids, less than 0.5 per cent of acids insoluble in 90 per cent alcohol at 20° was obtained. They melted at about 70°, which is analogous to the so-called isolation of "arachidic and lignoceric" acids from rapeseed oil by the "lead-ether" method reported by Ponzio[40] and Archbutt.[41] (At 25° crystallization of the solid acids, however, this precipitate was not found.) Having filtered off the precipitate, the solvent was expelled and the residue dried to constant weight.

Two samples of genuine American rape oils were examined and the yield of the solid acids was about 25 per cent. Upon analysis of this solid acid mixture, the following constants were obtained: (1) Melting point 35° (erucic acid 32°), (2) Mean molecular weight about 328 (erucic acid 338.45), (3) Iodine number 70°-73° (erucic acid 74.99).

Rape and other oils of the crucifereae group contain mainly glycerides of erucic, small amounts of rapic acid and glycerides of saturated acids. Magnesium soaps of rapic acids and the greater part of the saturated acids perhaps stay in 90 per cent alcohol solution, as inferred from the differences between the saponification numbers, iodine numbers of the insoluble acids from saponified oils and those of the acid obtained by the proposed method. Therefore the acid isolated above can be called, though with reserve, erucic acid. A confirmatory test can be performed by reducing it to behenic acid, melting point 84°, with an active catalyst in a suitable solvent. This will be investigated later.

In this connection, the author may mention that in 1911, when the price of linseed oil was very high, rape oil was frequently used to adulterate it. Since magnesium soaps from linseed oil, when thoroughly washed, give no residue of solid acids, the isolation of erucic and its reduction to behenic acid will afford a valuable method for detection of rape oil in linseed oil.

(2) Tung Oil: Magnesium soaps of all oils examined before are either entirely or almost entirely soluble in 90 per cent alcohol at the boiling temperature of the soap solution. The insoluble soaps precipitated out upon standing or cooling. The author was surprised to find that tung oil gave an abundance of white, elastic magnesium soaps insoluble even upon boiling under a reflux condenser for fifteen minutes. Since tung oil is said to contain the glycerides of mainly eleo-margaric acid, oleic acid and small amounts of saturated acids and since the magnesium soaps of the oleic acid and small amounts of saturated acids are soluble in boiling alcohol, the insoluble substance is probably, by process of elimination, magnesium eleo-margarate.

The insoluble magnesium soaps, while boiling hot, were filtered off, washed with the same hot solvent and decomposed with dilute hydrochloric acid. The acid liberated had a strong characteristic odor of tung oil, perhaps due to impurities present. The yield was about 20 per cent. It was soluble in 90 per cent alcohol and melted about 44°. After one or two days standing in the air, it readily absorbed oxygen and changed gradually to a dark brown resinous mass. This change was greatly accelerated at elevated temperatures.

IX. SUMMARY AND CONCLUSIONS

From the foregoing discussions and experimental results, the conclusions drawn are as follows:

1. The "lead-ether" method, the best current quantitative method in peanut oil analysis, has the following disadvantages: (1) It is too long and complex; (2) the separation of saturated and unsaturated lead soaps is not quantitative; (3) there is appreciable loss of arachidic and lignoceric acids due to the solubilities of their lead soaps in ether; (4) washing and decomposing the lead soaps is difficult; (5) it offers an inadequate and uneconomical method for preparing fairly large amount of arachidic and lignoceric acids or other solid acids of high molecular weights, if desired for special purpose; (6) fire risk and expense involved in using anhydrous ether are great; (7) it is difficult to keep the experimental conditions constant throughout; (8) cottonseed oil interferes; (9) consistent results are not always reproducible.

II. The great differential solubilities of the magnesium soaps in 90 per cent alcohol have been measured and it has been shown that magnesium soaps afford an excellent means for the separation of magnesium stearate, arachidate, lignocerate from magnesium oleate, linolate and linolenate.

III. The solubility of potassium stearate, lignocerate and oleate in 91.5 per cent (by weight) alcohol and the corresponding lead salts in ether have been carefully determined.

IV. The solubilities of stearic and lignoceric acids in three strengths of alcohol have been determined and it has been shown that a quantitative separation of arachidic and lignoceric acids from

other saturated acids can be accomplished with 90 per cent (by volume) alcohol after standing one night at 20° or 25° in a water thermostat.

V. A new quantitative determination of peanut oil is proposed, based first on separation of magnesium soaps of saturated and unsaturated acids in 90 per cent alcohol; second, followed by further separation of the characteristic acids of peanut; namely, arachidic and lignoceric by means of their comparative insolubility in 90 per cent alcohol.

VI. The proposed quantitative method has the following advantages: (1) It eliminates the experimental difficulties of the "lead-ether" method. (2) It minimizes the constant errors, like the solubilities of lead soaps in ether. (3) A direct and more rapid separation of saturated and unsaturated acids in peanut oil is accomplished and without the use of a second solvent. (4) Higher percentage yields of arachidic and lignoceric acids than those from the "lead-ether" method are obtained. (5) The results are reproducible to higher degrees of concordance. (6) Common vegetable oils, including cold drawn cottonseed oil, do not interfere with the quantitative determination at 20° or 25°, though one or two of them do so with the "lead-ether" method. (7) The method offers an excellent means for preparing large quantities of arachidic, lignoceric or other fatty acids of the same or higher molecular weights, if desired for research or technical purposes.

This proposed method is not intended for the separation of saturated from all unsaturated acids in all oils, since magnesium erucate and probably oleomargarate were found to be insoluble in 90 per cent alcohol.

VII. Genuine peanut oils from remote countries or localities widely separated differ not more than 0.5 per cent in their arachidic and lignoceric contents. Besides the scientific interest, this affords a just and accurate way for the determination of genuineness of the commercial peanut oils and their admixtures.

VIII. Arachidic and lignoceric acids separated from the titrated solution of saponification number gives, with the proposed method, an excellent qualitative and a semi-quantitative method for identifying peanut oils.

VIII. The proposed method further offers a very convenient means of isolating the characteristic acids from rape and tung oil for identifying these oils qualitatively.

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